

**Amendments to the Claims:**

This listing of claims will replace all prior versions and listings of claims in the application.

**Listing of Claims:**

1-29. (CANCELLED)

30. (CURRENTLY AMENDED) [A]~~The~~ method of ~~claim 88, coupling a disulfide bridge containing protein or peptide to a carrier comprising the following steps,~~

- a) ~~irradiating the protein or peptide to create a thiol group in the protein or peptide by disulfide bridge disruption, and~~
- b) ~~incubating the irradiated protein or peptide with a carrier capable of binding a thiol group and thereby obtaining a coupling,~~

~~— or~~

- a) ~~incubating the protein or peptide with a carrier capable of binding a thiol group, and~~
- b) ~~irradiating the protein or peptide in the presence of said carrier to create a thiol group in the protein or peptide by disulfide bridge disruption and thereby obtaining a coupling~~

wherein the carrier is an insoluble support whereon more than one disulfide-bridge-containing protein or peptide are coupled, each protein or peptide being coupled to said carrier through said created thiol group.

31. (PREVIOUSLY PRESENTED) A method according to claim 30, wherein the protein or peptide comprises more than one disulfide bridge.

32. (PREVIOUSLY PRESENTED) A method according to any one of claims 30 or 31, wherein said irradiation step comprises light of a wavelength that excites one or more aromatic amino acids.

33. (PREVIOUSLY PRESENTED) A method according to any one of claims 30 to 32, wherein said irradiation step comprises light of a wavelength that excites one specific aromatic amino acid.

34. (PREVIOUSLY PRESENTED) A method according to any one of claims 30 to 33, wherein said aromatic amino acid(s) is/are selected from tryptophan, tyrosine and phenylalanine.

35. (PREVIOUSLY PRESENTED) A method according to any one of claims 30 to 34, wherein the irradiation is performed by multi-photon excitation, preferably by two-photon excitation.

36. (PREVIOUSLY PRESENTED) A method according to any one of claims 30 to 34, wherein said irradiation comprises light with a wavelength of about 295nm, 275nm or 254nm.

37. (PREVIOUSLY PRESENTED) A method according to claim 34, wherein said aromatic amino acid is tryptophan.

38. (PREVIOUSLY PRESENTED) A method according to claim 36, wherein the wavelength is about 295nm.

39. (PREVIOUSLY PRESENTED) A method according to any one of claims 31 to 38, further comprising the steps of:

- a) verifying one or more disulfide bridges in said protein or peptide,
- b) identifying one or more aromatic amino acid residues being a spatial neighbour of said one or more disulfide bridges, for the transfer of excitation energy from said one or more aromatic amino acid to said one or more

disulfide bridges,

- c) selecting a wavelength which specifically excites one or more of said aromatic amino acid residues, thereby disrupting one or more of said disulfide bonds.

40. (PREVIOUSLY PRESENTED) A method according to claim 39 wherein the aromatic amino acid residue is within 10Å of the disulfide bridge.

41. (PREVIOUSLY PRESENTED) A method according to claim 40, wherein the plane of the dipole of the side-chain of the aromatic amino acid is not orthogonal to the plane of the disulfide bridge.

42. (PREVIOUSLY PRESENTED) A method according to claims 39-41, wherein the amino acid residues located within an 8Å radius of the indole ring of said aromatic amino acid residue are over-represented by amidic amino acid residues (Asn, Gln), as well as, short aliphatic amino acid residues (Gly, Ala, Val) and/or long aliphatic amino acid residues (Leu, Ile) by at least 1 fold, and under-represented by charged amino acids (His, Lys, Arg)(Asp, Glu) and proline residues by at least 1 fold.

43. (PREVIOUSLY PRESENTED) A method according to any one of claims 30 to 38, wherein said protein or peptide is irradiated in the presence of a free aromatic amino acid.

44. (PREVIOUSLY PRESENTED) A method according to any one of claims 30 to 43, wherein said coupling is an immobilization on said support.

45. (PREVIOUSLY PRESENTED) A method according to claim 44, wherein said immobilization is spatially controlled.

46. (PREVIOUSLY PRESENTED) A method according to claim 45, wherein said support is a derivatised support that is capable of binding a thiol group.
47. (PREVIOUSLY PRESENTED) A method according to claim 46, wherein said support comprises a thiol group or a disulfide bridge.
48. (PREVIOUSLY PRESENTED) A method according to claim 47, wherein the support comprises a spacer.
49. (PREVIOUSLY PRESENTED) A method according to any one of claims 30 to 48, wherein the protein or peptide can furthermore be released from the carrier by irradiating the protein or peptide to create a thiol group in the protein or peptide by disulfide bridge disruption.
50. (PREVIOUSLY PRESENTED) A method according to claim 46, wherein said support comprises gold.
51. (PREVIOUSLY PRESENTED) An insoluble support comprising one or more protein or peptide coupled by the method of any one of claims 30 to 50.
52. (PREVIOUSLY PRESENTED) An insoluble support according to claim 51, wherein the coupled protein or peptide are specifically oriented.
53. (PREVIOUSLY PRESENTED) An insoluble support according to claim 52, wherein the support is selected from the group consisting of an electronic chip, slide, wafer, resin, well, tube, microarray and membrane.
54. (PREVIOUSLY PRESENTED) An insoluble support according to claim 53,

wherein the support comprises a material selected from the group consisting of topaz, polystyrene, polyethylene, polyester, polyetherimide, polypropylene, polycarbonate, polysulfone, polymethylmethacrylate, poly(vinylidene fluoride), silicone, diamond, quartz and silica, silicium, metal, nylon, nitrocellulose, agarose, cellulose and ceramic.

55. (PREVIOUSLY PRESENTED) An insoluble support according to any one of claims 51 to 54, wherein the protein or peptide coupled to the support are spatially controlled.

56. (PREVIOUSLY PRESENTED) Use of an insoluble support according to any one of claims 51 to 55 for a bio-functional reaction.

57. (PREVIOUSLY PRESENTED) Use of an insoluble support according to claim 56, wherein said biofunctional reaction is selected from the group consisting of a biosensor, chromatography, immunodetection, enzyme assay, nucleotide binding detection, protein-protein interaction, protein modification, carrier targeting and protein targeting.

58. (PREVIOUSLY PRESENTED) Use of the method according to any one of claims 30 to 50 for the production of a bio-sensor or a protein/peptide microarray.

59. (PREVIOUSLY PRESENTED) Use of an insoluble support according any of claims 51 to 55 in a diagnostic or biosensor kit.

60. (CURRENTLY AMENDED) [A]The method of claim 88, coupling a disulfide bridge containing protein or peptide to a carrier comprising the following steps,

- e) —irradiating the protein or peptide to create a thiol group in the protein or peptide by disulfide bridge disruption, and
- d) —incubating the irradiated protein or peptide with a carrier capable of binding

~~a thiol group and thereby obtaining a coupling,~~

~~— or~~

e) ~~— incubating the protein or peptide with a carrier capable of binding a thiol group, and~~

d) ~~— irradiating the protein or peptide in the presence of said carrier to create a thiol group in the protein or peptide by disulfide bridge disruption and thereby obtaining a coupling~~

wherein the carrier is soluble and capable of being decoupled from said protein or peptide by irradiation.

61. (PREVIOUSLY PRESENTED) A method according to claim 60, wherein the protein or peptide comprise more than one disulfide bridge.
62. (PREVIOUSLY PRESENTED) A method according to claim 60 or 61, wherein said irradiation step comprises light of a wavelength that excites one or more aromatic amino acids.
63. (PREVIOUSLY PRESENTED) A method according to any one of claims 60 or 62, wherein said irradiation step comprises light of a wavelength that excites one specific aromatic amino acid.
64. (PREVIOUSLY PRESENTED) A method according to any one of claims 60 to 63, wherein said aromatic amino acid(s) is/are selected from tryptophan, tyrosine and phenylalanine.
65. (PREVIOUSLY PRESENTED) A method according to any one of claims 60 to 63, wherein the irradiation is performed by multi-photon excitation, preferably by two-photon excitation.

66. (PREVIOUSLY PRESENTED) A method according to any one of claims 60 to 63, wherein said irradiation comprises light with a wavelength of about 295nm, 275nm or 254nm.

67. (PREVIOUSLY PRESENTED) A method according to claim 66, wherein said aromatic amino acid is tryptophan.

68. (PREVIOUSLY PRESENTED) A method according to claim 66, wherein the wavelength is about 295nm.

69. (PREVIOUSLY PRESENTED) A method according to any one of claims 60 to 68, further comprising the steps of:

- a) verifying one or more disulfide bridges in said protein or peptide,
- b) identifying one or more aromatic amino acid residues being a spatial neighbour of said one or more disulfide bridges, for the transfer of excitation energy from said one or more aromatic amino acid to said one or more disulfide bridges,
- c) selecting a wavelength which specifically excites one or more of said aromatic amino acid residues, thereby disrupting one or more of said disulfide bonds.

70. (PREVIOUSLY PRESENTED) A method according to claim 69 wherein the aromatic amino acid residue is within  $10\text{\AA}$  of the disulfide bridge.

71. (PREVIOUSLY PRESENTED) A method according to claim 70, wherein the plane of the dipole of the side-chain of the aromatic amino acid is not orthogonal to the plane of the disulfide bridge.

72. (PREVIOUSLY PRESENTED) A method according to claims 69-71, wherein the amino acid residues located within an 8Å radius of the indole ring of said aromatic amino acid residue are over-represented by amidic amino acid residues (Asn, Gln), as well as, short aliphatic amino acid residues (Gly, Ala, Val) and/or long aliphatic amino acid residues (Leu, Ile) by at least 1 fold, and under-represented by charged amino acids (His, Lys, Arg)(Asp, Glu) and proline residues by at least 1 fold.

73. (PREVIOUSLY PRESENTED) A method according to any one of claims 60 to 68, wherein said protein or peptide is irradiated in the presence of a free aromatic amino acid.

74. (PREVIOUSLY PRESENTED) A method according to any one of claims 60 to 73, wherein the protein or peptide may be released from the carrier by irradiating the protein or peptide to create a thiol group in the protein or peptide by disulfide bridge disruption.

75. (PREVIOUSLY PRESENTED) A method according to claim 60, wherein said carrier comprises a peptide, a protein or another biomolecule.

76. (PREVIOUSLY PRESENTED) A coupled carrier coupled to one or more proteins or peptides obtainable by the method of any one of claims 60 to 75.

77. (PREVIOUSLY PRESENTED) A carrier according to claim 74, wherein the coupled protein or peptide are specifically oriented.

78. (PREVIOUSLY PRESENTED) A coupled carrier according to claim 76 or 77, wherein the one or more protein or peptide is selected from the group consisting of an enzyme, transcription factor, protein domain, binding protein, antigen and



immunoglobulin.

79. (PREVIOUSLY PRESENTED) A coupled carrier according to claim 78, wherein said immunoglobulin is a F(ab) fragment.

80. (PREVIOUSLY PRESENTED) A coupled carrier according to claim 88, wherein said enzyme is selected from the group consisting of cutinase, chymosin, glucose oxidase, lipase, lysozyme, alkaline phosphatase and plasminogen.

81. (PREVIOUSLY PRESENTED) A coupled carrier according to claim 76, wherein the protein or peptide comprises a drug or a prodrug.

82. (PREVIOUSLY PRESENTED) Use of a coupled carrier according to any one of claims 76 to 81 for drug delivery

83. (PREVIOUSLY PRESENTED) A method of delivering of a drug or prodrug to a patient comprising the following steps of:

- (a) providing a carrier coupled to one or more proteins or peptides according to any one of claims 76 to 81
- (b) administering the carrier-coupled protein or peptide to a patient
- (c) irradiating the carrier-coupled protein or peptide to create a thiol group in the molecule by disulfide bridge disruption and thereby releasing the protein or peptide from the carrier.

84. (PREVIOUSLY PRESENTED) A method according to claim 83, wherein the carrier is a pharmaceutical drug.

85. (PREVIOUSLY PRESENTED) A method according to claim 83, wherein the

protein or peptide is a drug or a prodrug.

86. (PREVIOUSLY PRESENTED) A method of predicting a disulfide bridge containing protein or peptide capable of disruption by irradiation for use in the method of claims 30-50 or method of claims 60-75, comprising the steps of:

- a) identifying and selecting a disulfide bridge containing protein or peptide, and
- b) identifying and selecting a protein or peptide selected in (a), further comprising an aromatic amino acid residue within  $10\text{\AA}$  of said disulfide bridge, and
- c) identifying and selecting a protein or peptide selected in (b), wherein the plane of the dipole of the side-chain of said aromatic amino acid is not orthogonal to the plane of said disulfide bridge.

87. (PREVIOUSLY PRESENTED) A method according to claim 86, further comprising the step of: identifying and selecting a protein or peptide selected in (b) or (c), wherein the amino acid residues located within an  $8\text{\AA}$  radius of the indole ring of said aromatic amino acid residue are over-represented by amidic amino acid residues (Asn, Gln), as well as, short aliphatic amino acid residues (Gly, Ala Val) and/or long aliphatic amino acid residues (Leu, Ile) by at least 1 fold, and under-represented by charged amino acids (His, Lys, Arg)(Asp, Glu) and proline residues by at least 1 fold.

88. (NEW) A method of coupling a disulfide bridge containing protein or peptide to a carrier comprising the following steps:

- a) irradiating the protein or peptide to create a thiol group in the protein or peptide by disulfide bridge disruption, and
  - b) incubating the irradiated protein or peptide with a carrier capable of binding a thiol group and thereby obtaining a coupling,
- or

- a) incubating the protein or peptide with a carrier capable of binding a thiol group, and
- b) irradiating the protein or peptide in the presence of said carrier to create a thiol group in the protein or peptide by disulfide bridge disruption and thereby obtaining a coupling,

wherein the carrier is an insoluble support whereon more than one disulfide-bridge-containing protein or peptide are coupled, each protein or peptide being coupled to said carrier through said created thiol group; or

wherein the carrier is soluble and capable of being decoupled from said protein or peptide by irradiation.